Acute toxicity and relationship between metabolites and ecotoxicity during the biodegradation process of non-ionic surfactants: fatty-alcohol ethoxylates, nonylphenol polyethoxylate and alkylpolyglucosides


ABSTRACT

The toxicity values of fatty-alcohol ethoxylates, nonylphenol polyethoxylate, and alkylpolyglucosides have been determined by applying assays with luminescent bacteria. Also, the relation between metabolites and ecotoxicity during the biodegradation process has been determined. The biodegradation tests were carried out according to the OECD 301 E test for ready biodegradability. In these tests a solution of the surfactant, representing the sole carbon source for the microorganisms, was tested in a mineral medium, inoculated and incubated under aerobic conditions in the dark. The toxicity of surfactants is related to their molecular structure (Quantitative Structure Activity Relationships, QSAR). For the alkylpolyglucosides, toxicity expressed as EC50 is related with the critical micelle concentration (CMC), the hydrophilic-lipophilic balance (HLB) of the surfactant, and the hydrophobic alkyl chain (R). The results indicate that toxicity increased as the CMC decreased and as the hydrophobicity increased and R rose. For fatty-alcohol ethoxylates, parameters characteristic studied have been HLB, number of units of ethylene oxide and the alkyl chain length. Relationships found are in agreement with the fact that increasing the alkyl chain length leads to a lower EC50, whereas increasing ethoxylation leads to a lower toxicity. An analysis of the behaviour of the toxicity and HLB again indicates that the toxicity was greater for surfactants with a smaller HLB. The evolution of the toxicity was studied over the biodegradation process, expressed as a percentage of inhibition. For all the non-ionic surfactants assayed, except for the nonylphenol polyethoxylate, a major decline was found in toxicity during the first days of the biodegradation assay and at all the concentrations tested.

Key words | biodegradation, bioluminescent organisms, non-ionic surfactants, toxicity

INTRODUCTION

Many types of bioassays are available to establish the toxicity levels of compounds for aquatic organisms, but many of these tests are also time-consuming and not routinely applicable. Although several bioassays using microorganisms have been described, most of the bacterial screening tests have been based on measurements of luminiscence, because they are rapid, reproducible, simple to use and are cost-effective. One of these methods is the well known Microtox® (Farré et al. 2001). These assays provide a rapid response and, while not flawless, serve to compare different contaminants. In addition to providing assays with lyophilized bacteria, these assays present high degrees of reproducibility, as demonstrated with inter-laboratory tests. The characteristics of speed, reliability, and normalization of the toxicity results by bioassays of toxicity with luminescent bacteria make them ideal for
gathering data on toxicity, which can be compared and statistically studied for establishing correlations between toxicity as well as the chemical structure and/or different properties of the compounds assayed. Studies have been made relating ecotoxicity with temperature, pH, exposure time, hydrophobicity, etc. \(\text{Ribó 1987}\), with CMC \(\text{Sanchez-Leal 1995}\), and with interfacial properties \(\text{Rosen et al. 2001}\).

Non-ionic surfactants are generally complex substances, not well defined single chemical structures but are mixtures containing multiple structurally similar chemicals. The ecotoxicity of a complex substance can depend heavily on the shape of the distribution of its different components. For example, for surfactants, ecotoxicity typically increases logarithmically with a linear increase in alkyl chain length \(\text{Boeije et al. 2006}\). In such cases of nonlinearity, the most highly toxic components have an impact on toxicity that is disproportionate to their molar abundance, whereas their impact on the calculation of the substance’s average structure is proportionate to molar abundance. Hence, it is possible that a complex mixture will significantly have different ecotoxicity with respect to the single substance representing its average structure.

The enormous worldwide use of surfactants, which are generally dumped into water systems, requires them to be as innocuous as possible for the environment: low toxicity and easily biodegraded. The comparison of different types of surfactants by ecotoxicological tests with luminescent bacteria together with biodegradability can also determine the choice for including them in detergent formulas, taking into account also their effectiveness in the wash.

It is important to relate the toxicity of surfactants with their molecular structure, quantitative structure-activity relationships (QSAR). Although a substantial body of data is available on the aquatic toxicity of various surfactants, there are few reported QSARs which correlate such data. The best-known relationship is that using the octanol/water coefficient partition \(\log K_{ow}\) \(\text{Roberts 1991}\), and perhaps the best-known equation is that of Könemann for the toxicity of chemically unreactive aliphatic and aromatic compounds in 14-day LC50 tests on guppies \(\text{Könemann 1981}\). Sometimes the \(\log K_{ow}\) value is not sufficient to model the biological response. Uppgard et al. approached the problem with multivariate quantitative structure activity (M-QSAR), for ethoxylated fatty alcohols \(\text{Uppgard et al. 2000a}\) and alkylpolyglucosides \(\text{Uppgard et al. 2000b}\).

An expression that related \(EC_{50}\) values measured with \(D. magna\) with alkyl chain length \(R\) and EO units has been proposed by \(\text{Wong et al. 1997}\):

\[
EC_{50} = 10^{-0.38 R + 0.1 EO - 1.77}
\]  

Also, for the estimation of acute \(D. magna\) toxicity, \(\text{Boeije et al. (Boeije et al. 2006)}\) proposed the following two equations:

\[
EC_{50} = 10^{-0.58 \log K_{ow} - 2.70}
\]

\[
EC_{50} = 10^{-0.32 R + 0.12 EO - 2.26}
\]

For continued advancement in the search for relationships between toxicity and structural parameters in the field of surfactants, in the present work the ecotoxicity assay with luminescent bacteria is applied to different commercial surfactants widely used at present: fatty-alcohol ethoxylates (FAEs), nonylphenol polyethoxylate (NPEO), and alkylpolyglucosides (APGs). The FAEs are composed of a hydrophobic alkyl chain \(R\) with a terminal ethoxylate (EO) chain with \(n\) ethoxylated units (Figure 1). NPEOs are composed of a hydrophobic alkyl chain \(R\), linear or branched, a benzenic ring and a terminal ethoxylate (EO) chain with \(n\) ethoxylated units (Figure 1). The APGs are prepared on the basis of renewable raw materials, namely

![Structure of different surfactants.](https://iwaponline.com/wst/article-pdf/59/12/2351/435368/2351.pdf)
(starch/sugar) and fatty alcohols (vegetable oils). They also belong to the group of nonionic surfactants and can be described in terms of an acetal structure (Figure 1). The fatty alcohol radicals have 8 to 16 carbon atoms, and DP the average number of glucose units per alkyl radical, ranging between 1 to 2.

Also, a study is made of the monitoring of ecotoxicity during the biodegradation process of the above-mentioned surfactants.

**MATERIALS AND METHODS**

The following surfactants were used in this study: fatty-alcohol ethoxylates with the general formula R(−O−CH₂−CH₂)n−OH, listed in Table 1, a nonylphenol polyethoxylate (R−C₆−H₄−O−(CH₂−CH₂−O)₉.₅H), both supplied by Kao Corporation, and alkylpolyglucosides manufactured by Henkel and supplied by Sigma.

In these experiments, the measurement was taken with the measuring system LumiStox® 300, which consists of an incubation unit and an instrument for measuring bioluminescence (Bulich 1986). The toxicity measurement is based on the luminous intensity of the marine bacteria of the strain *Vibrio fisheri* NRRLB11177 after a certain exposure time to a toxic substance. The luminescent bacteria, from the supplier Hach Lange, were dehydrated and frozen at −18°C and were reactivated with the suspension supplied by Hach Lange. The assay conditions were pH = 7.0 and ClNa concentration of 2%. All the measurements were duplicated for incubation times of 15 and 30 min. When necessary, the sample was filtered prior to the assay.

The toxicity value was measured as EC₅₀ or EC₂₀, which are, respectively, the surfactant concentrations that inhibit 50 and 20% after 15 and 30 min of exposure.

In addition, during the biodegradation process of the surfactant, a toxicity analysis was made of the evolution of the metabolites generated. The measurements were made following the same procedure indicated above. In this case, the results could not be expressed in terms of concentration, as the samples were of unknown composition. Therefore, to evaluate the toxicity during the biodegradation process the toxicity value of the sample is expressed as a (%) of inhibition, and the variation in toxicity during the biodegradation assay as the variation of the inhibition percentage (Jurado et al. 2004). The inhibitory effect of the samples assayed was calculated for an incubation time of 15 min.

The biodegradation tests were carried out according to the OECD 301 E test for ready biodegradability (OECD 1993). A solution of the surfactant, representing the sole carbon source for the microorganisms, was tested in a mineral medium, inoculated and incubated under aerobic conditions in the dark. The procedure consists of introducing 1.2 litres of surfactant solution (for which the biodegradability is to be determined) into a 2-litre Erlenmeyer flask and inoculating the solution with 0.5 mL of

![Table 1](https://iwaponline.com/wst/article-pdf/59/12/2351/435368/2351.pdf)
water from a secondary treatment of a sewage-treatment plant (STP) that operates with active sludge. This water sample is a mixed aerobic culture of faecal microorganisms including, for the most part, total coliforms, faecal coliforms and enterococcus. The Erlenmeyer flask is plugged with a cotton stopper and left in darkness in a thermostatically controlled chamber at 25°C. The constant rotary speed of the orbital shaker (125 sweep/min) provides the necessary aeration. The surfactant solution is prepared by dissolving the desired quantity of surfactant in the nutrient solution. The biodegradation assays are carried out at different initial concentrations of surfactant: 15, 20, 25, and 50 mg/L. These concentrations are used because lower concentrations give unmeasurable toxicity values, and we wished to study the effect of concentration on toxicity. The samples from biodegradation tests are taken every 8 h approximately to analyse the toxicity.

RESULTS AND DISCUSSION

The toxicity was determined for different surfactants: FAEs, NPEO, and APGs. The initial concentrations of the surfactant were between 100 and 500 mg/L, depending on the surfactant assayed.

The initial values of luminous intensity measured were corrected by a factor that takes into account the natural decrease in luminous intensity, even in the absence of the toxic sample:

\[ f_k = \frac{I_0(0)}{I_0(t)} \]  

with \( I_0(0) \) and \( I_0(t) \) being the readings of luminous intensity in the well containing concentration 0 at time 0 and \( t \) (Equation 4).

The percentage of inhibition (inhibitory effect) was calculated by the expression:

\[ H_t = \frac{(I_0(c) - I_t(c))}{I_0(c)} \times 100 \]  

where

\[ I_0(c) = f_k I_0(c) \]  

with \( f_k \) being the average correction factor of the control samples, \( I_0(c) \) and \( I_t(c) \) being readings of luminous intensity in the well containing concentration \( c \) at time 0 and \( t \).

The Gamma function, the ratio between the light intensity lost by the bacterial solution and that remaining after exposure to the toxic sample, can be evaluated by the equation:

\[ G_t = \frac{H_t}{100 - H_t} = \frac{f_k I_0(c) - I_t(c)}{I_t(c)} \]  

From the results, a linear relationship can be deduced between the function \( G \) and the concentration of the surfactant used, in the following form:

\[ \log(c) = b \cdot \log(G) + \log(a) \]  

The values of \( EC_{20} \) and \( EC_{50} \), expressed as mg/L, are calculated, giving \( G \) values of 0.25 and 1, respectively. The results for the different surfactants assayed are presented in Table 2, in decreasing order of toxicity, for incubation times of 15 and 30 min.

The results indicate for all the non-ionic surfactants assayed the toxicity presented \( EC_{50} \) (15 min) values of 1.24

| Table 2 | Values of \( EC_{20} \) (95% CI) and \( EC_{50} \) (95% CI) in mg/L for different surfactants |
|---------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Surfactant | \( EC_{20} \) (15 min) | \( EC_{50} \) (15 min) | \( EC_{20} \) (30 min) | \( EC_{50} \) (30 min) |
| FINDET 1214N/16 | 0.47 (0.45–0.49) | 1.24 (1.22–1.25) | 0.43 (0.41–0.44) | 1.42 (1.40–1.43) |
| FINDET 1015 | 0.72 (0.61–0.82) | 2.01 (1.90–2.02) | 0.71 (0.60–0.72) | 2.21 (2.10–2.22) |
| FINDET 10/18 | 1.37 (1.18–1.55) | 4.76 (4.57–4.77) | 1.20 (1.01–1.21) | 4.80 (4.61–4.81) |
| FINDET 1214N/23 | 5.54 (5.51–5.57) | 12.67 (12.64–12.68) | 5.93 (5.90–5.94) | 13.26 (13.23–13.27) |
| FINDET 1618A/23 | 6.22 (5.45–6.98) | 37.18 (36.41–37.18) | 5.81 (5.04–5.81) | 35.64 (34.87–35.64) |
| GCP 215 | 10.12 (8.11–12.13) | 29.05 (27.04–29.07) | 6.21 (4.20–6.21) | 25.59 (23.58–25.60) |
| FINDET 1618A/18 | 12.27 (10.32–14.21) | 146.53 (144.58–146.54) | 13.16 (11.21–13.20) | 85.78 (83.83–86.74) |
| NPEO | 29.29 (20.06–38.51) | 160.64 (151.41–160.64) | 26.53 (17.30–26.53) | 162.90 (153.67–163.50) |
to 160.64 mg/L, with the most toxic being the FAE with the commercial name FINDET 1214N/16 and the least NPEO.

The alkylpolyglucosides had EC₅₀ (15 min) values of 14 to 29 mg/L (Table 2), those of the shortest carbon chain and lowest glucose content being the least toxic (GLUCOPON 215). García et al. (1997) used Daphnia magna to determine the acute toxicity of commercial alkylpolyglucosides with chain sizes of C₉–C₁₆, finding that the mixtures of the most hydrophobic alkylpolyglucosides—i.e. of the longest carbon chains—were the most toxic for the test used, registering EC₅₀ (30 min) values of 7 to 16 mg/L. These results coincide with our findings in assays performed with Photobacterium fischeri (Figure 2a). Similar studies also showed that the homologous of alkylpolyglucosides of the longest alkyl chain presented the highest ecotoxicity values (Steber et al. 1995).

In addition, the toxicity values (EC₅₀, 15 min) were plotted against the HLB of the three alkylpolyglucosides used (Figure 2b) showing that toxicity fell as HLB rose. Another parameter characteristic of surfactants is their critical micelle concentration (CMC); on representing the variation in toxicity against the CMC (Figure 2c), a potential relation was also found between toxicity and the CMC, toxicity falling as the CMC of the surfactant rose. The results are in agreement with the data in the literature, even using other test microorganisms (Uppgard et al. 2000).

For FAEs, the relationships between toxicity and different parameters characteristic of these surfactants have also been analysed. In this case, the relationships found for toxicity were HLB, number of units of ethylene oxide and the alkyl chain length.

Figure 3 plots the toxicity values (EC₅₀, 15 min) versus HLB.

The relation found and the corresponding coefficients of determination are:

$$EC_{50}(15 \text{ min}) = 10^{0.223 \text{HLB }^2 - 2.06} \text{ (mg/L)}$$

$$R^2 = 0.933$$  (9)

The influence of alkyl chain length and the number of units of ethylene oxide (EO) has been analysed using an
expression similar to the one proposed by Wong et al. (1997) and Boeije et al. (2006). The relation found with our data is:

\[
EC_{50}(15\text{ min}) = 10^{-0.192 R + 0.151 EO + 1.88} \text{ (mg/L)}, \quad R^2 = 0.979
\] (10)

Figure 4 shows the experimental results versus values calculated with this equation.

Analysing the overall results, we conclude that contributions of EO units in toxicity in this R/EO QSAR is almost identical to that in Boeije et al. (2006). Different contribution is found for alkyl chain length. This fact may be explained by the different organism used in the assays: V. fisheri versus D. magna.

An analysis of the behaviour of the toxicity and HLB again indicates that the toxicity was greater for surfactants with a smaller HLB. This fact has also been treated in the literature, as numerous studies seeking to establish structure-activity relationships have concluded that the FAEs with greater hydrophobicity – that is lower HLB – show higher toxicity values (Wong et al. 1997). Therefore, this characteristic parameter of all surfactants could be taken as a reference for predicting FAEs toxicity. These results are consistent with those proposed by Boeije et al. (Boeije et al. 2006), who established a relationship between the EC\textsubscript{50} found with toxicity assays using D. magna and log \(K_{ow}\) (Equation 2).

In any case, both relations are in agreement with the fact that increasing the alkyl chain length leads to a lower EC\textsubscript{50}, whereas increasing ethoxylation leads to lower toxicity.

To eliminate any doubt concerning the environmental lability of surfactants, a study was made of the evolution of the toxicity over the biodegradation process. In this way, it can be confirmed whether metabolites of biodegradation are more toxic than the original surfactant, or on the other hand whether the surfactants studied have acceptable environmental biodegradability—that is, the toxicity of the biodegradation metabolites is less than that of the initial surfactant and that the surfactants are not only primary biodegradable or mineralizable.

To study the effect of concentration on the toxicity, the biodegradation assays were carried out at different initial concentrations of surfactant: 15, 20, 25 and 50 mg/L.

For each sample, the inhibition of luminescence was measured after 15 min of incubation, expressing the results as a (%) of inhibition. Figure 5 shows an example of the toxicity results during the biodegradation process for the surfactant FINDET 1618A/18 for an initial concentration of 50 mg/L together with its biodegradation curve (Figure 5a). Analogous results were found for the rest of the FAEs and concentrations. Figure 5b and 5c present the toxicity results during the biodegradation process for the alkylpolyglucoside GLUCOPON 650 and for NPEO, at starting concentrations of 20 and 25 mg/L, respectively.

Finally, Figure 5d shows the effect of the concentration on the variation in the inhibition (%) over the biodegradation process of the FAE FINDET 1214N/23, reflecting that as the surfactant concentration augmented in the assay, more time was needed to reach null toxicity values.

All the non-ionic surfactants tested, except for NPEO, diminished significantly in toxicity during the first few days.
of the biodegradation assay and at all the concentrations tested. For NPEO, the toxicity during the biodegradation process peaked, thus confirming that the biodegradation metabolites were more toxic than the starting compound, as indicated in the literature (Marcomini et al. 2000). Therefore, in general, we can state that, except for the case of NPEO, the metabolites produced over the biodegradation process presented far lower toxicity than the original surfactant, classifying them as having acceptable environmental biodegradability.

CONCLUSIONS

The toxicity values of non-ionic surfactants were determined for fatty-alcohol ethoxylates, nonylphenol polyethoxylate and alkylpolyglucosides, applying assays with luminescent bacteria with the measurement system Lumistox® 300. The results showed EC$_{50}$ (15 min) values of 1.24 to 160.64 mg/L, with the most toxic being the fatty-alcohol ethoxylate FINDET 1214N/16 (with the longest carbon chain and lowest degree of ethoxylation) and the least toxic nonylphenol polyethoxylate. For predicting the environmental behaviour of a surfactant, it is important to relate the toxicity of the surfactants with their molecular structure (QSAR). For alkylpolyglucosides, a relationship was investigated between toxicity CMC, HLB, and R. The toxicity declined as the CMC rose but augmented for lower HLB values and for greater length of the carbon chain.

For fatty-alcohol ethoxylates, the relations between EC$_{50}$ and HLB, and EO together with R were studied. It was concluded that the toxicity increased as the parameter HLB decreased, coinciding with the literature and with results found for alkylpolyglucosides. The contribution of EO and R are also consistent with the literature and indicate that increasing the alkyl chain length leads to a lower EC$_{50}$, whereas increasing ethoxylation leads to lower toxicity.

Finally, the evolution of the toxicity, expressed as a percentage of inhibition, was studied over the biodegradation process.
process. For all the non-ionic surfactants assayed, except for nonylphenol polyethoxylate, a sharp decline in toxicity was observed during the first few days of the biodegradation assay and at all the concentrations tested. For nonylphenol polyethoxylate it was found that the toxicity during the biodegradation process peaked, thus confirming that the biodegradation metabolites were more toxic than the starting compound.

REFERENCES


